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is exacerbated by non-random pairing, the demonstration of such a strong relationship between parent and offspring inbreeding coefficients has important implications for the way that we interpret the causes of variation in natural populations. For example, if parents that inbreed are themselves inbred, then this may lead to over-estimation of the magnitude of inbreeding depression, because part of the estimated effect of inbreeding in offspring might be attributable to inbreeding in parents [14,15]. The magnitude of inbreeding depression is of considerable importance for our understanding of the genetic architecture of quantitative traits [16], and the evolution of dispersal, kin recognition and other potential inbreeding avoidance mechanisms [7]. Secondly, a parent–offspring inbreeding correlation would lead to the overestimation of the size of additive genetic effects for those traits that are more strongly influenced by inbreeding.

Worryingly, the traits that show the strongest inbreeding depression tend to be those on which selection acts most strongly [17], and for which understanding the genetic basis of traits provides the biggest challenge [18,19]. It may turn out that the very high parent–offspring resemblance for inbreeding found by Reid *et al.* [6] is a special characteristic of small island populations with low rates of immigration, but until that is established, biologists interested in understanding the causes of variation in wild populations should spend more time considering the relationships between inbreeding, relatedness and population structure in wild populations.

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Calcium Signaling: Double Duty for Calcium at the Mitochondrial Uniporter

Uptake of Ca^{2+} by mitochondria serves as a regulator of a number of important cellular functions, including energy metabolism, cytoplasmic Ca^{2+} signals, and apoptosis. Recent findings reveal that the process of Ca^{2+} uptake by the mitochondrial uniporter is itself regulated by Ca^{2+} in a temporally complex manner.

James W. Putney, Jr.[1] and Andrew P. Thomas[2]

The history of Ca^{2+} handling by mitochondria is a classic story of rags to riches (or rather riches to rags to riches) [1]. In the 1970s to early 1980s, mitochondria were thought to be prime sources of signaling Ca^{2+} in non-excitable cells. However, this idea fell into disfavor with the finding that, at the Ca^{2+} levels expected in either resting or activated cells, the relatively high Km for uptake should preclude significant Ca^{2+} accumulation by mitochondria [2], and ultimately with the finding that the signal for intracellular Ca^{2+} release, inositol trisphosphate (IP_3), clearly mobilized Ca^{2+} from endoplasmic reticulum [3]. Biochemical studies of mitochondrial Ca^{2+} content and the regulation of mitochondrial Ca^{2+}-sensitive enzymes indicated that mitochondria were more likely to be a target for Ca^{2+} signaling rather than a source [4,5]. When Rizzuto *et al.* [6] made the first direct *in situ* measurements of mitochondrial Ca^{2+}, it was clear that receptor-activated Ca^{2+} signals caused rapid and large Ca^{2+} signals in the mitochondrial matrix. It soon became apparent that mitochondria are capable of

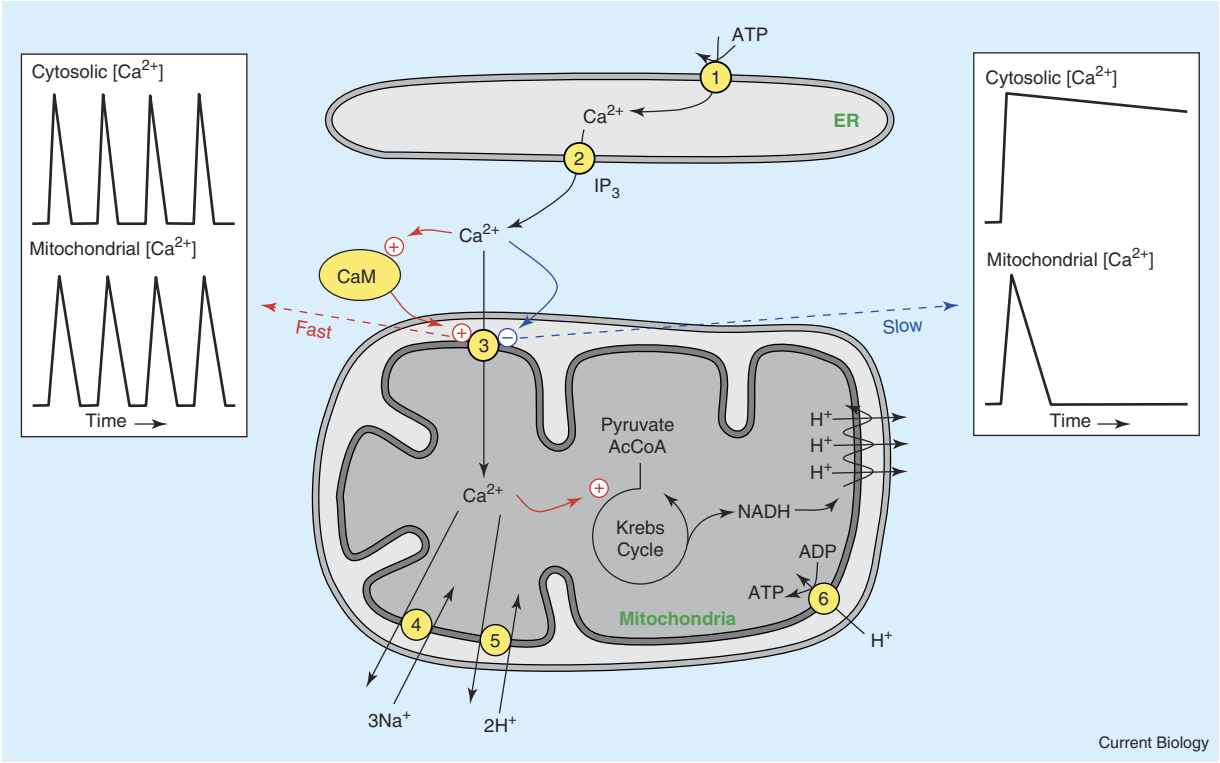

Figure 1. Regulation of mitochondrial Ca^{2+} uptake.

Close apposition between mitochondria and endoplasmic reticulum (ER) Ca^{2+} release sites facilitates Ca^{2+} uptake into the mitochondria. A fast activation of the uniporter, mediated by calmodulin (CaM), enhances mitochondrial Ca^{2+} accumulation. A slower Ca^{2+}-dependent inactivation of the uniporter serves to limit mitochondrial Ca^{2+} uptake during a prolonged rise of cytosolic Ca^{2+}. The fast activation allows the mitochondrial matrix [Ca^{2+}] to closely follow changes in the cytosolic [Ca^{2+}], as occurs during cytosolic Ca^{2+} oscillations (left inset). The slower inactivation process limits mitochondrial Ca^{2+} uptake when the elevation of cytosolic Ca^{2+} is more sustained (right inset). (1) SERCA Ca^{2+} pump, (2) IP_3 receptor Ca^{2+} release channel, (3) Ca^{2+} uniporter, (4) mitochondrial Ca^{2+}/Na^+ exchanger, (5) mitochondrial Ca^{2+}/H^+ exchanger, (6) ATP synthetase. The mitochondrial respiratory chain is shown as 3 cycles of proton pumping from the matrix.

accumulating Ca^{2+} during signaling processes because they are positioned very near to either the sites of intracellular release (by IP_3, for example) or sites of entry across the plasma membrane (for example, through store-operated or voltage activated channels; for a review see [7]). Ca^{2+} activates several key enzymes in the mitochondrial matrix to enhance ATP production, and this provides an important mechanism for synchronizing energy production with the energy demands of Ca^{2+}-activated processes during cell stimulation (excitation–metabolism coupling) [4,5,8]. In addition to serving as a target of Ca^{2+} signaling, the uptake of Ca^{2+} by mitochondria has important feedback effects to help shape cytosolic Ca^{2+} signals. This can occur through buffering of bulk cytosolic Ca^{2+} changes, but is most pronounced in the intimate intracellular 'synaptic' regions where mitochondria are in close proximity to Ca^{2+} release sites. Thus, rapid accumulation of Ca^{2+} can prevent or temper influences of Ca^{2+} on intracellular or plasma membrane channels [9,10]. Alternatively, in some instances mitochondrial Ca^{2+} uptake serves to compartmentalize Ca^{2+} signaling in appropriate cellular domains, a concept termed "firewall" from studies of pancreatic acinar cells [11]. In addition, under certain conditions, uptake of Ca^{2+} into mitochondria initiates a key step in the process of apoptosis through activation of the mitochondrial permeability transition pore, permitting escape of cytochrome c and other pro-apoptotic factors to the cytoplasm [12].

On the surface, mitochondrial Ca^{2+} handling seems rather simple. Uptake occurs through a channel termed a uniporter (from the Peter Mitchell nomenclature) and the rate of uptake depends upon driving force; this is considerable, as the process of electron transport in normally respiring mitochondria generates extremely negative transmembrane potentials across the inner mitochondrial membrane. This would ultimately lead to huge and potentially toxic levels of accumulated Ca^{2+} in the mitochondrial matrix were it not for the action of separate mitochondrial Ca^{2+} efflux pathways that are also coupled to the proton motive force developed by the respiratory chain. Thus, the inner mitochondrial membrane has a $Ca^{2+}/2H^+$ exchanger and/or a $Ca^{2+}/3Na^+$ exchanger analogous to that found in the plasma membrane. However, these efflux pathways can become saturated with high matrix Ca^{2+} loads, such

that sustained rapid Ca^{2+} influx can still lead to mitochondrial Ca^{2+} overload.

In a report in a recent issue of *Current Biology*, Moreau *et al.* [13] reveal that the process of Ca^{2+} accumulation undergoes complex regulation by Ca^{2+} itself. They measured mitochondrial matrix Ca^{2+} concentration directly by loading the mitochondria of permeabilized mast cells (a rat basophilic leukemia line) with a fluorescent Ca^{2+} indicator. The uptake of Ca^{2+} was significantly reduced by inhibitors of calmodulin, suggesting that a Ca^{2+}–calmodulin-mediated process is necessary for activation of the uniporter. This finding is consistent with an earlier observation that calmodulin antagonists impede the penetration of Mn^{2+} into mitochondria and that brief pulses of cytosolic Ca^{2+} can facilitate mitochondrial Ca^{2+} uptake [14]. Surprisingly, Moreau *et al.* [13] found that Ca^{2+} also appeared to inhibit its own uptake. Thus, uptake of Ca^{2+} due to addition of 100 μM Ca^{2+} was substantially impaired if preceded by exposure to 10 μM Ca^{2+}. In contrast to the sensitization of mitochondrial Ca^{2+} uptake, the Ca^{2+}-dependent inactivation was not sensitive to calmodulin blockers. The ability of Ca^{2+} to inactivate the uniporter may correspond to the phenomenon of desensitization of mitochondrial Ca^{2+} uptake suggested in earlier studies [15,16]. The uniporter appeared to be similarly sensitive to both activation and inhibition by Ca^{2+}, with apparent Kds in the 10–20 μM range. This concentration range raises the standard mitochondrial problem of whether such Ca^{2+} concentrations are seen by the mitochondria during physiological signaling. In agreement with an earlier study [17], Moreau *et al.* show close associations between mitochondria and the endoplasmic reticulum, and go on to show that when Ca^{2+} is released from the endoplasmic reticulum by IP_3 there is a much more efficient transfer of Ca^{2+} to the mitochondria than that seen with exogenous Ca^{2+} addition. Thus, in the mast cell line, Ca^{2+} released by IP_3 is readily sensed by mitochondria, probably due to close apposition of release and uptake sites. There is ample evidence in the literature for such associations between mitochondria and the endoplasmic reticulum [18]. Interestingly, mitochondria have been shown to be somewhat mobile in cells, while the endoplasmic reticulum is relatively fixed; Ca^{2+} inhibits mitochondrial mobility, thus providing a mechanism to retain mitochondria at specific Ca^{2+} signaling sites [19].

The fact that both the activation and inactivation mechanisms have similar Ca^{2+} sensitivities would seem problematic for significant accumulation of Ca^{2+} in the mitochondrial matrix. However, when Moreau *et al.* [13] examined the kinetics of inactivation, it was found that inactivation occurred with a time constant of 17 seconds, while for uptake the time constant is about 6 seconds. This pattern of rapid activation and slower inactivation is reminiscent of myriad biological signaling patterns; most notably, as pointed out by Moreau *et al.* [13], of the biphasic regulation of Ca^{2+} discharge from the endoplasmic reticulum through the IP_3 receptor channel. In most cell types, physiological Ca^{2+} signaling occurs not by sustained elevations in cytoplasmic Ca^{2+}, but through repetitive bursts of rising intracellular Ca^{2+}, sometimes referred to as Ca^{2+} oscillations, with durations ranging from less than a second to several seconds [20]. The delayed inactivation mechanism will thus permit mitochondrial matrix Ca^{2+} to closely track these global signals, but will prevent excessive Ca^{2+} accumulation if the intracellular Ca^{2+} elevation is prolonged (Figure 1). For example, hormone-induced cytosolic Ca^{2+} oscillations in hepatocytes are closely paralleled by mitochondrial Ca^{2+} oscillations, whereas high levels of hormone that yield a sustained cytosolic Ca^{2+} increase cause only a transient spike of mitochondrial Ca^{2+} [8]. Thus, the Ca^{2+}-dependent activation and inactivation of mitochondrial Ca^{2+} uptake [13] may help to tune mitochondrial responses to oscillatory Ca^{2+} signals, while filtering out persistent Ca^{2+} elevations that would have pathological consequences. Thus, once considered a simple Ca^{2+} accumulating depot, mitochondria have since proven to be elegantly controlled players in the complex network of intracellular Ca^{2+} signaling.

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